AN EXPERIMENTAL STUDY ON THE EFFECTS OF BREEDER DIETS ON NEWBORN SEAHORSES (*Hippocampus guttulatus*)

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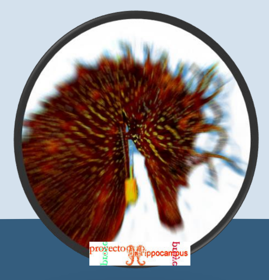

INTRODUCTION

Aquaculture represents a valid alternative to wild captured seahorses but *ex-situ* production still needs to face several biological and technical challenges (Cohen et al., 2016; Planas et al., 2017). Low spawning quality and juvenile survival rates are the most critical factors for optimizing commercial seahorse production. Reproduction represents a key aspect in breeding success, and broodstock nutrition is one of the most important factors in reproductive achievements. Even though the growth of adult *Hippocampus guttulatus* fed on *Artemia* may be satisfactory, mysidaceans have been considered an excellent prey for the on-growing and breeding of some seahorse species (e.g. *H. abdominalis* and *H. hippocampus*) (Olivotto et al., 2011). In this study, we assessed the effects of *Artemia* and mysidaceans in newborn. We addressed the following questions: (1) How experimental diets are reflected in general features and biochemical composition of *H. guttulatus* newborn?, (2) Do mixed diets perform better than monospecific diets?, and (3) Does *H. guttulatus* follow a mixed income-capital breeding strategy? The main objectives of the study were (1) to propose a breeder's diet enhancing reproduction performance in the species, and (2) to ascertain when and how long the diet should be administered to take effect.

MATERIALS AND METHODS

Three homogeneous (size, weight) groups of adult seahorses (reared or wild collected) were maintained in captivity and fed on a mixed diet including enriched *Artemia* and mysidaceans. From the onset of the breeding period (5.5 months; 20 March – 5 Oct 2017) each group was fed on one of the following diets: Unenriched adult *Artemia* (Diet A), unenriched adult *Artemia* and mysidaceans (Diet AM) and mysidaceans (Diet M). Newborns (0 DAR) were counted, measured (standard length), weighted (dry weight), sampled for total lipids, fatty acid and electronic microscopy analyses, and reared on copepods and *Artemia* nauplii. Survival was recorded at day 7 (7 DAR). The data set was submitted to Principal Component Analysis (PCA).

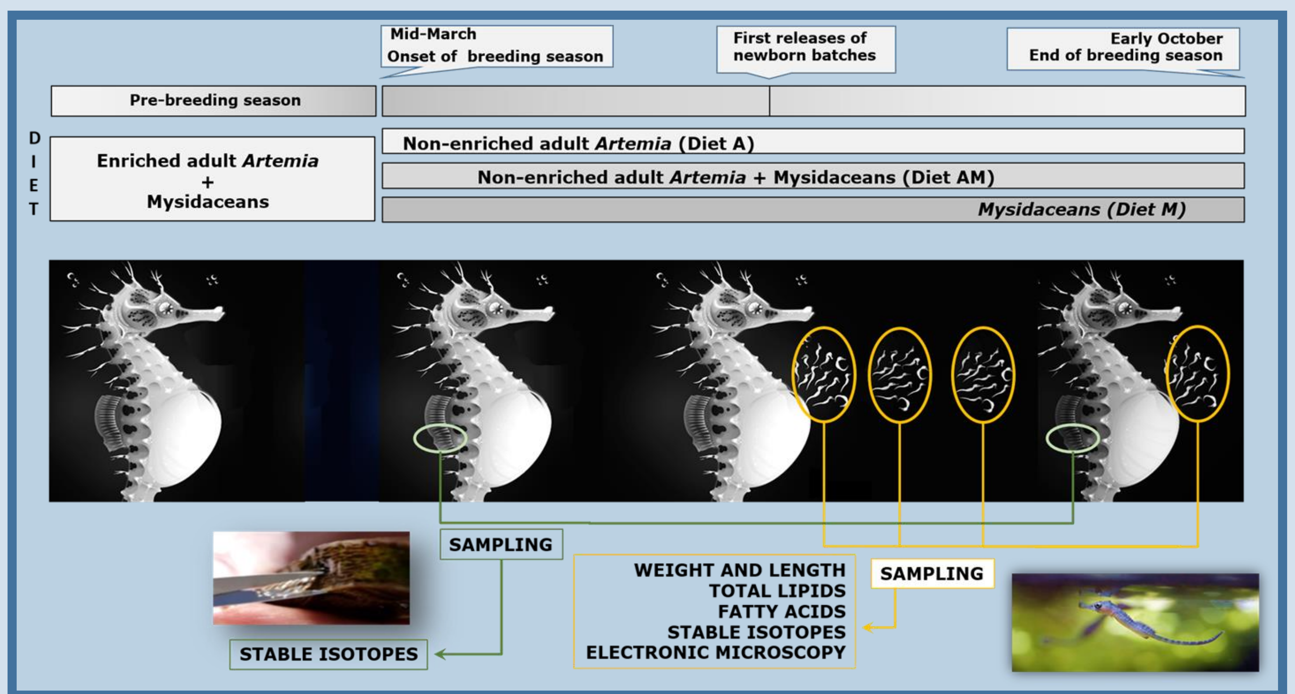

Table 1. Breeding performance.

Diet	Batches produced	Total juveniles	Batch size mean ± sd	Length (mm) mean ± sd	Dry weight (mg) mean ± sd	Survival (%)
A	4	534	$134^{a} \pm 83$	$12.9^{a} \pm 0.7$	$0.60^{a} \pm 0.16$	$94.0^{a} \pm 5.3$
AM	7	1,416	$202^{a} \pm 82$	$14.7^{b} \pm 0.7$	$0.74^{a} \pm 0.11$	$86.9^{a} \pm 9.5$
M	3	248	$84^{a} \pm 72$	$14.1^{b} \pm 0.2$	$0.73^{a} \pm 0.06$	$61.4^{a} \pm 19.2$
W	1	-	210	14.1	0.78	94.9

RESULTS AND DISCUSSION

- The breeding performance was enhanced in parents fed on the mixed diet AM, suggesting a better nutritional condition than in groups A and M (Table 1). The use of only mysidaceans (Diet M) provided the worse results.
- Significant differences were noticed in newborn characteristics and fatty acid profiles across dietary groups (Figure 1) and along the breeding season (Figure 2), especially in n-3 HUFA (DHA).
- Some abnormal muscle ultrastructure (disarrangement of myofibrils and interruption of Z bands) in newborn released by males from diet A would indicate some nutritional deficiencies in unenriched *Artemia*.
- We hypothesize that the following stages can be recognized along the whole breeding period (Figure 2):
  - Stage 1 (mixed capital-income sources; 100 – 120 days since the onset of the breeding period): Progressive changes as a consequence of the diet. Resources would firstly originate from nutrients gained prior to breeding (capital breeding), followed by an increasing use of experimental dietary sources (income breeding).
  - Inflexion period (income sources): An inflexion period following stage 1 shows clear differences between treatment and the use exclusively of resources from the experimental diet.
  - Stage 2 (income sources and exhaustion of body reserves): Maximal divergences between treatments were reached, notably by the end of the stage. The trend followed by certain variables (e.g. DHA, DHA/EPA) in late newborn batch released suggests a more or less intense exhaustion of body resources, accompanied by decreases in survivals at 7 DAR and newborn size. This statement is also supported by the trends in newborn isotopic signatures (Planas et al., 2020).

CONCLUSIONS

Monospecific diets including *Artemia* or mysidaceans very likely suffered nutritional deficiencies, which were reflected in morphological alterations of the muscle tissue (diet A) and abnormal early mortalities in newborn (diet M). The mixed diet AM (unenriched *Artemia* and mysidaceans) provided the highest overall breeding success and reasonable fatty acid profiles, and better resembled newborn from a wild male. However, special attention deserve the progressive changes in fatty acid profiles along the breeding season, resulting in a decrease in newborn performance. Considering the time required for a diet to be reflected in newborn, we recommend that *Hippocampus guttulatus* breeders be fed on a high quality diet at least 3 months in advance of the onset of the breeding period.

REFERENCES

Cohen et al. 2016. Seahorse aquaculture, biology and conservation: Knowledge gaps and research opportunities. Rev Fish Sci Aquac 25, 1–12.
Olivotto et al. 2011. Advances in breeding and rearing marine ornamentals. J World Aquacult Soc 42, 135–166.
Planas et al. 2017. Seahorses and pipefish. In: Calado R, Olivotto I, Planas M, Holt GJ (eds) Marine ornamental species aquaculture, Wiley Blackwell, West Sussex, UK, 299-326 pp.
Planas et al. 2020. Effect of diet on breeders and inheritance in syngnathids: application of isotopic experimentally derived data to field studies. Mar. Ecol. Prog. Ser., Advance View https://doi.org/10.3354/meps13315.

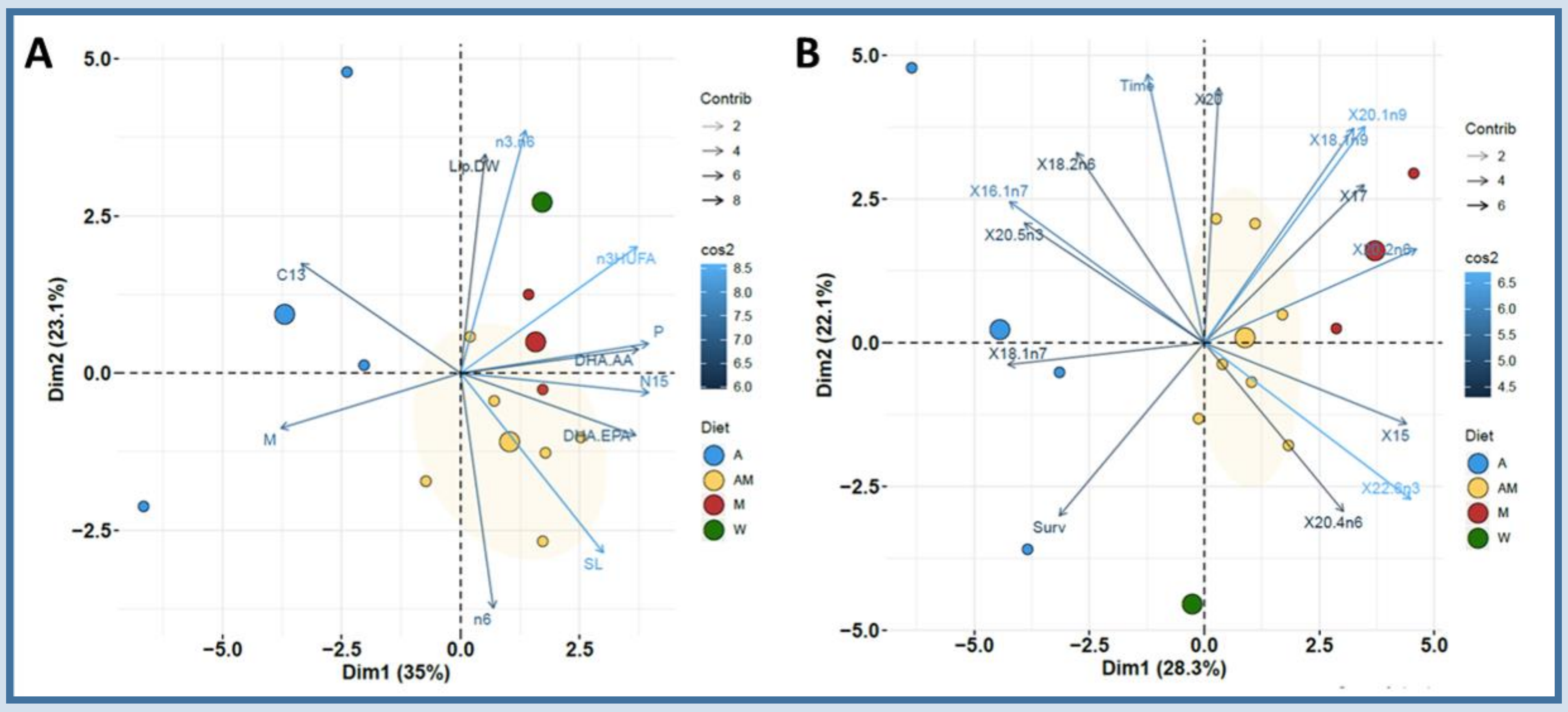

Fig. 1: PCA on newborn released by wild (W) or cultivated (A, AM and M) seahorses. Only the variables with the highest contributions (cos2 > 0.6) on factors 1 and 2 maps are shown. Variables: Days elapsed since the onset of the breeding period (Time), SL, W, Survival at 7 DAR (Surv), total C (C.), total N (N.), C/N (C.N), δ13C (C13), δ15N (N15), total lipids (Lip.DW), saturated FA (S), monounsaturated FA (M), polyunsaturated FA (P), n-3 HUFA, n-3 FA, n-6 FA, n-3/n-6 (n3.n6), DHA/EPA and DHA/AA (left), and individual FA (right).

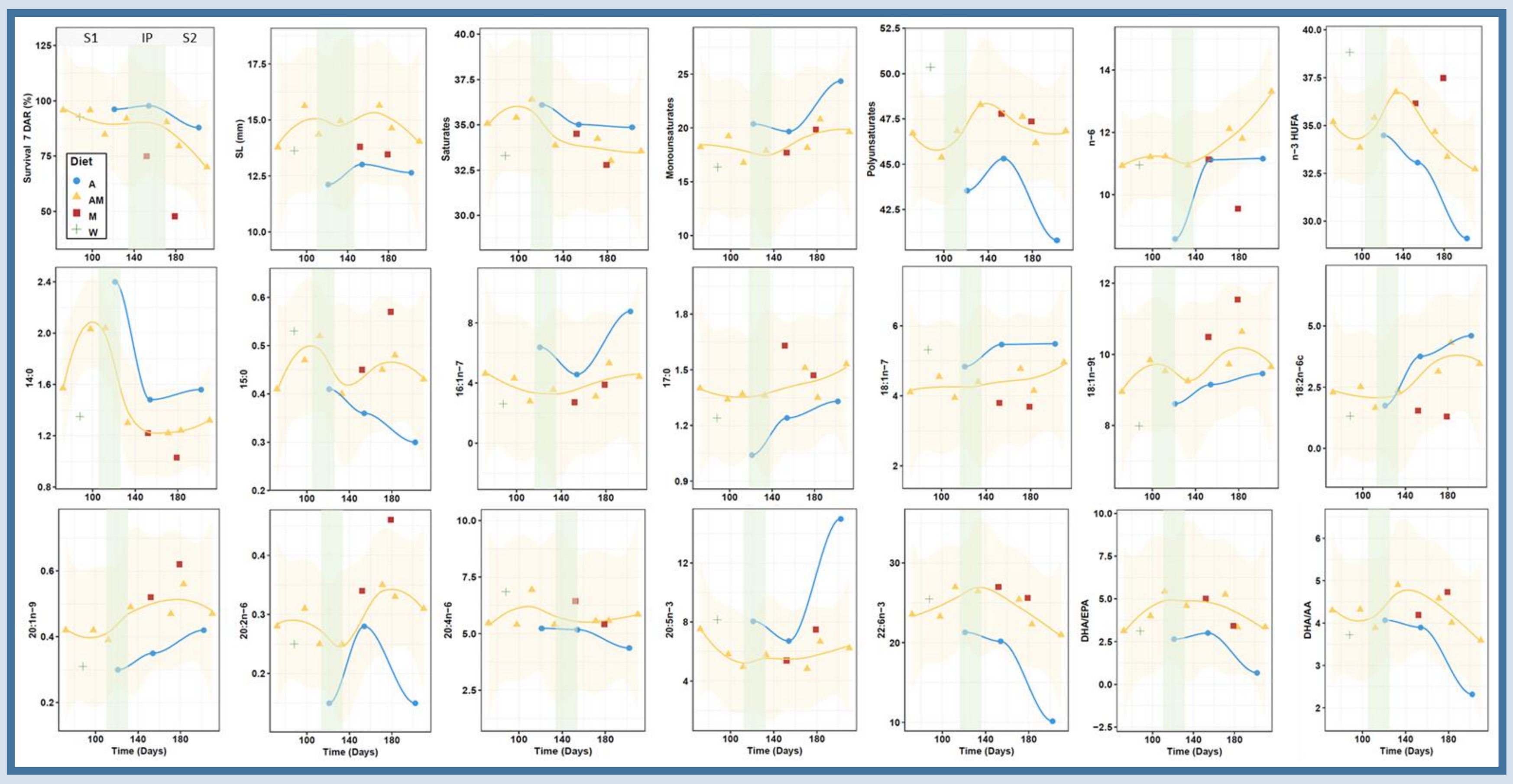

Fig. 2. Progressive changes in survival at 7 DAR, length (SL), fatty acids (area percentage), and FA ratios along the breeding period in newborn released by seahorses (Wild and groups A, AM and M). Smoothed trends are shown for group AM (95% confidence intervals also shown) and A. Green shadowed areas show the inflexion period (IP) indicating a remarkable change. Stages: S1 (Stage 1), IP (Inflexion period) and S2 (Stage 2).

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BIOETHICS: Animal capture, handling and sampling were conducted in compliance with all bioethics standards on animal experimentation of the Spanish Government (Real Decreto 1201/2005, 10th October 2005) and the Regional Government Xunta de Galicia (REGA ES360570202001/15/FUN/BIOL.AN/MPO01)